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IN VITRO SOMATIC EMBRYOGENESIS IN TWO MAJOR RATTAN SPECIES: *CALAMUS MERRILLII* AND *CALAMUS SUBINERMIS*

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SUMMARY

Occurrence of somatic embryogenesis in *in vitro* cultures of *Calamus merrillii* and *Calamus subinermis*, two major large-caned rattan species, was scientifically demonstrated for the first time. Tissue responsiveness varied markedly according to the species and the type of primary explants used when initiated on 10.4–31.2 μ*M* picloram-enriched Murashige and Skoog callus induction media. In *C. merrillii*, within 6 wk after inoculation, 84% of the leaf and 90% of the zygotic embryo explants produced friable embryogenic calluses, by contrast with those formed by 74% of the root explants. In *C. subinermis*, callogenesis was observed only 6 mo. after inoculation in 68% of root and 48% of zygotic explants. Leaf explants did not respond at all. Only root-derived calluses developed into nodular embryogenic structures. Irrespective of these initial differences, the further steps of the somatic embryogenesis developmental pattern was similar for both species. Histological analyses established that callus formation took place in the perivascular zones, and could give rise to embryogenic isolated cells from which the proembryos were derived. Reducing the picloram concentration stimulated the maturation process resulting ultimately in the germination of somatic embryos that exhibited bipolar development, despite an apparent lack of starch and protein reserves. The somatic embryo-derived plantlets of *C. merrillii*, overall more prone to somatic embryogenesis than *C. subinermis* in the given conditions, were successfully acclimatized to outdoor conditions.

Key words: asexual embryogenesis; histology; *in vitro* culture; palms.

INTRODUCTION

Rattans are spiny climbing palms belonging to the large subfamily *Calamoideae* and grow in tropical rainforests. These species are largely represented in South-East Asia. *Calamus merrillii* is endemic to the Philippines, whereas *Calamus subinermis* occurs naturally along the coast of northern Borneo and of Palawan (Fernando, 1990; Dransfield and Manokaran, 1993). They are both large-caned and multiple stem rattan species, although *C. subinermis* may also be solitary (Dransfield and Manokaran, 1993), and are very much prized along with the single-stemmed *Calamus manan* for furniture making. The high market demand has accounted for an overexploitation of the species, especially during the past two decades, which, in turn, has resulted in a dramatic depletion of natural resources (Umali-Garcia and Villena-Sanches, 1990). In order to counteract this critical situation, *C. merrillii* and *C. subinermis* have been intensively planted over the past 15 yr within industrial forestry projects, particularly in Sabah, East Malaysia (Nasi and Monteuuis, 1992; Goh et al., 1997) and in Mindanao, southern Philippines (Umali-Garcia and Villena-Sanches, 1990; PCARRD, 1991).

Despite seeds having been the most widely used means for propagating *C. merrillii* and *C. subinermis*, vegetative propagation can be useful for improving the genetic quality of the planting stock (Goh et al., 1997). Owing to the limitations of horticultural techniques for vegetatively mass propagating these two species (Aziah and Manokaran, 1985; Umali-Garcia and Canlas-Mendoza, 1996), efforts have been devoted to tissue culture methods. *In vitro* shoot proliferation has been proven to be feasible when using shoot tips excised from *in vitro*-germinated or nursery seedlings (Umali-Garcia, 1985), notwithstanding limited multiplication rates and high variability in responses among explants (Goh et al., 1997). Within this context, somatic embryogenesis is worth special attention considering its numerous applications, including clonal propagation of mature selected genotypes. This technology has already been successfully applied to palm species of major economical importance such as oil palm, date palm, coconut, and more recently the rattan *C. manan* (Goh et al., 1999). The fact that somatic embryogenesis can be initiated from tissues other than the shoot apex is a real asset, especially for single-stemmed species in which removing the shoot tip results in the death of the donor plant. Somatic embryogenesis has already been attempted by Umali-Garcia and Villena-Sanches (1990) on different rattan species, including *C. merrillii*; however, due to the problems encountered, embryo rescue and multiple shoot formation were preferred (Umali-Garcia and Canlas-Mendoza, 1996). Protocols developed within our project through callus formation gave rise to plantlets of *C. merrillii*,

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and for the first time, at least as far as we are aware, of *C. subinermis* (Goh et al., 1997). To ascertain the somatic origin of the embryos obtained and to better understand the whole process with a view to improving its efficiency, histological analyses were undertaken.

Materials and Methods

Somatic embryogenesis. Three different kinds of explants were used to induce embryogenic callus formation for both *C. subinermis* and *C. merrillii*. These were (1) root tip fragments (1–2 cm in length) excised from the most terminal part of the primary roots of 3-mo.-old seedlings germinated *in vitro* (Goh et al., 1997); (2) portions (1.5 cm average size) of young unopened leaves from 6 to 8 mo. old *in vitro* seedlings; and (3) zygotic embryos excised from fruits soon after field harvesting. Root and leaf explants were inoculated for callus formation without any further disinfection treatments. The zygotic embryos, 1–2 mm and 2–3 mm in size for *C. subinermis* and *C. merrillii*, respectively, were excised in contamination-free conditions from seeds. These seeds had been surface-sterilized by 10 min soaking in a 10% (v/v) domestic bleach solution (5.25% NaOCl) followed by three sterile ultrapure water washes. The primary culture medium for all explant types consisted of Murashige and Skoog (1962) macro- and micronutrients supplemented with 100 mg l^{-1} *myo*-inositol, 500 mg l^{-1} casein hydrolysate, 2 mg l^{-1} glycine, 1 mg l^{-1} thiamine, 1 mg l^{-1} pyridoxine-HCl, 1 mg l^{-1} nicotinic acid, 30 g l^{-1} sucrose and 2.5, 5 or 7.5 mg l^{-1} (10.4, 20.7, 31.1 μM, respectively) picloram. After pH adjustment to 5.6–5.8 with 1 *N* KOH and the addition of 7 g l^{-1} 'high gel strength' Sigma agar (cat. no. A-9799), 12.5 ml of the medium were dispensed into 21 × 150 mm glass culture tubes prior to sterilization by autoclaving at 120°C and 95 kPa for 20 min. Fifty to 200 explants of each type were individually inoculated per culture tube, for both species on the three concentrations of picloram tested in the primary culture medium. The samples were maintained in these culture tubes covered with polypropylene caps, in total darkness at 27 ± 2°C and relative humidity (RH) of about 90%. The calluses obtained were subcultured once onto the same culture medium as described above. Thereafter, they were divided and transferred several times at 4–8-wk intervals onto a maturation medium with picloram at lower concentrations than in the previous callus induction medium, that is 1, 2.5 or 5 mg l^{-1} (4.1, 10.4 or 20.7 μM, respectively).

Somatic embryo maturation and germination. The embryos that developed from calluses were subcultured again at 4–8-wk intervals onto the same basal culture medium but without any growth regulator. The embryos were considered germinated once the plumule appeared. From this stage, they were then transferred under a 16-h light/dark photoperiod (50–60 μmol m^{-2} s^{-1}, 'TLD 36W/84 Philips' fluorescent lamps) at 28/22 ± 2°C, and RH of about 70%. The development of the first leaf from the shoot apex together with the elongation of the radicle occurred soon after. This marked the completion of the germination process.

Histology. The specimens selected for histological analysis were fixed for 24 h in a phosphate buffer (0.2 *M*, pH 7.2) containing 2% *para*-formaldehyde, 1% glutaraldehyde and 1% caffeine to precipitate oxidized phenol compounds *in situ*. After dehydration through a graded alcohol series, samples were embedded in LKB resin (Leica Rueil-Malmaison, France) and cut into 3-μm thick sections. For each specimen studied, sections were double-stained with periodic acid Schiff (PAS) and Naphthol blue black (NBB). PAS stained starch reserves and cell walls red (Martoja and Martoja, 1967), and NBB specifically stained soluble or reserve proteins blue-black (Fisher, 1968).

Results

Calamus subinermis. Callus formation appeared to be mainly dependent upon the type of primary explant used, without any significant influence of the different picloram concentrations tested. Overall, leaf explants did not respond at all while 48% of the zygotic embryo cultures produced calluses after 6–8 wk (Table 1). However, these calluses became spongy in texture with a fuzzy surface, and several weeks later turned brown, most likely due to phenolic oxidations. These were then discarded. Regardless of the concentration of picloram used (10.4, 20.7 or 31.1 μM), root tips were the most responsive type of explants tested, as on average 68% of the cultures produced calluses after a minimum of 6 mo. when placed on these picloram-enriched media (Table 1). Primary calluses occurred on apices of primary or secondary roots (Fig. 1A). They also proliferated along the cut surface of the root as protuberances of the central cylindrical zone (Fig. 1B). In the early phase of the process, these calluses looked soft and whitish and would remain in this state for a long period before new proliferation or differentiation of friable and granular yellowish protuberances could appear (Fig. 1C). When transferred to a medium containing a lower amount of picloram (for instance, from 10.4 to 4.1 or 2 μM), these friable masses would revert to the previous stage and become soft-whitish. However, if left untransferred for another 5–8 wk, the friable calluses would again proliferate, evolving towards a mixture of creamy and yellowish nodular calluses that characterized a further advancement in maturity.

TABLE 1

COMPARATIVE PROPORTIONS OF CALLUSES OBTAINED AFTER 6 MO. OF CULTURE FOR *C. SUBINERMIS* AND 6 WK FOR *C. MERRILLII*

Species	Type of primary explant		
	Zygotic embryo	Leaf portion	Root tip
C. subinermis	96/200 = 48% b	0/50 = 0% a	34/50 = 68% c
C. merrillii	180/200 = 90% b	42/50 = 84% ab	37/50 = 74% a

The three types of primary explants were tested for the two species in the same experimental conditions combining the three concentrations (10.4, 20.7 or 31.1 μM) of picloram tested.

For each species, letters distinguish proportions which are significantly different at $P = 0.05$ (χ^2-Pearson's test).

Upon transfer onto fresh media or media with a lower picloram level, a progressive maturation occurred, generally after another 6 mo. in culture. The nodular calluses became glossy, and eventually various structures with globular, rounded or elongated shapes were observed (Fig. 1D). The most promising type of embryos with potential development into plantlets was dome-shaped with a pointed apex, succulent in texture, and translucent-looking. Over time, structures representing the shoot would emerge from the cluster of potential plantlets. The shoot tip was greenish and soon became leaf-like. Most of these prospective plantlets appeared to be stunted with no elongation of the shoot-like structures, despite the various treatments tried so far. These included culture on growth regulator-free media with (2 g l^{-1}) and without activated charcoal, and even on media containing various concentrations of gibberellic acid (0.5, 1.0 and 3.0 mg l^{-1} or 1.4, 2.9 and 8.7 μM, respectively), which instead of promoting shoot elongation, induced the formation of greenish and vigorous roots.

Overall, only approximately 5% of the root tip-derived calluses produced *in vitro* somatic embryos, out of which only a few reached the suitable stage of development (Fig. 1E) for acclimatization to outdoor conditions (data not shown).

Histological observations established that primary callus originated mainly from perivascular zones of root explants (Fig. 1F). The soft and whitish calluses consisted of highly differentiated cells with large vacuoles and few starch reserves. Over time, the proportion of

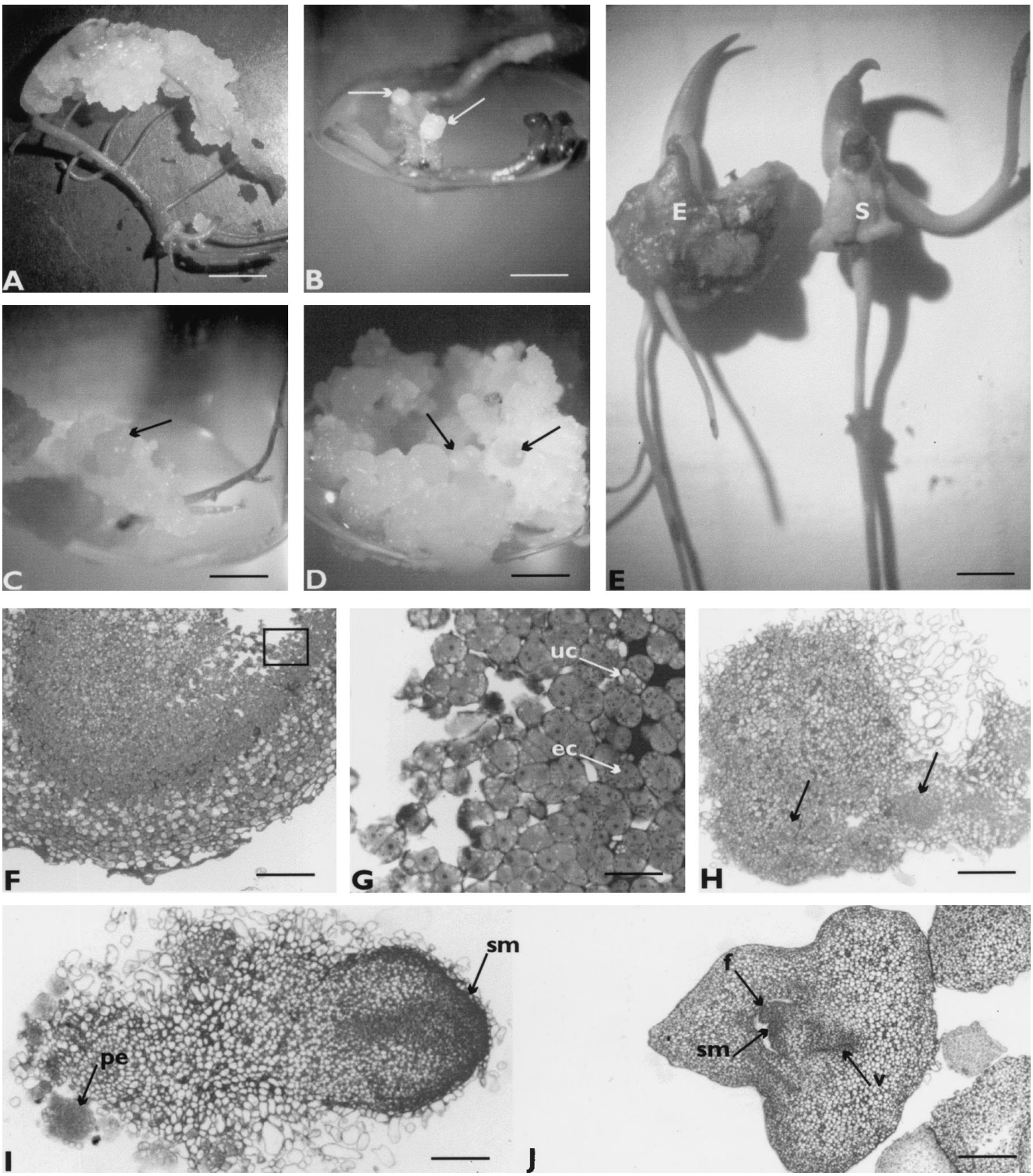

FIG. 1. *C. subinermis*. A, Primary calluses produced by root tips (*bar* = 3.3 mm). B, Primary calluses (arrows) emerging from the central cylindrical zone of a longitudinally sliced root (*bar* = 3.3mm). C, Friable yellowish callus (arrow) coming out from the whitish and soft primary callus (*bar* = 3.3mm). D, Translucent proembryo-like structures (arrows) (*bar* = 2.7 mm). E, Somatic embryo-derived plantlet (E) and seedling (S) used as control, ready for acclimatization to *ex-vitro* conditions (*bar* = 1.9 cm). F, Callus originating from root tip perivascular cells; box magnified in (G) (*bar* = 125 μm). G, Magnification of (F) box showing undifferentiated (uc) and embryogenic cells (ec) (*bar* = 31 μm). H, Proembryogenic masses (arrows) (*bar* = 250 μm). I, Proembryo (pe) development showing in the most advanced stage shoot apical meristem (sm) formation (*bar* = 210 μm). J, Longitudinal section of a developing somatic embryo showing the shoot apical meristem (sm), a foliar primordium (f) and the vascular axis (v) (*bar* = 275 μm).

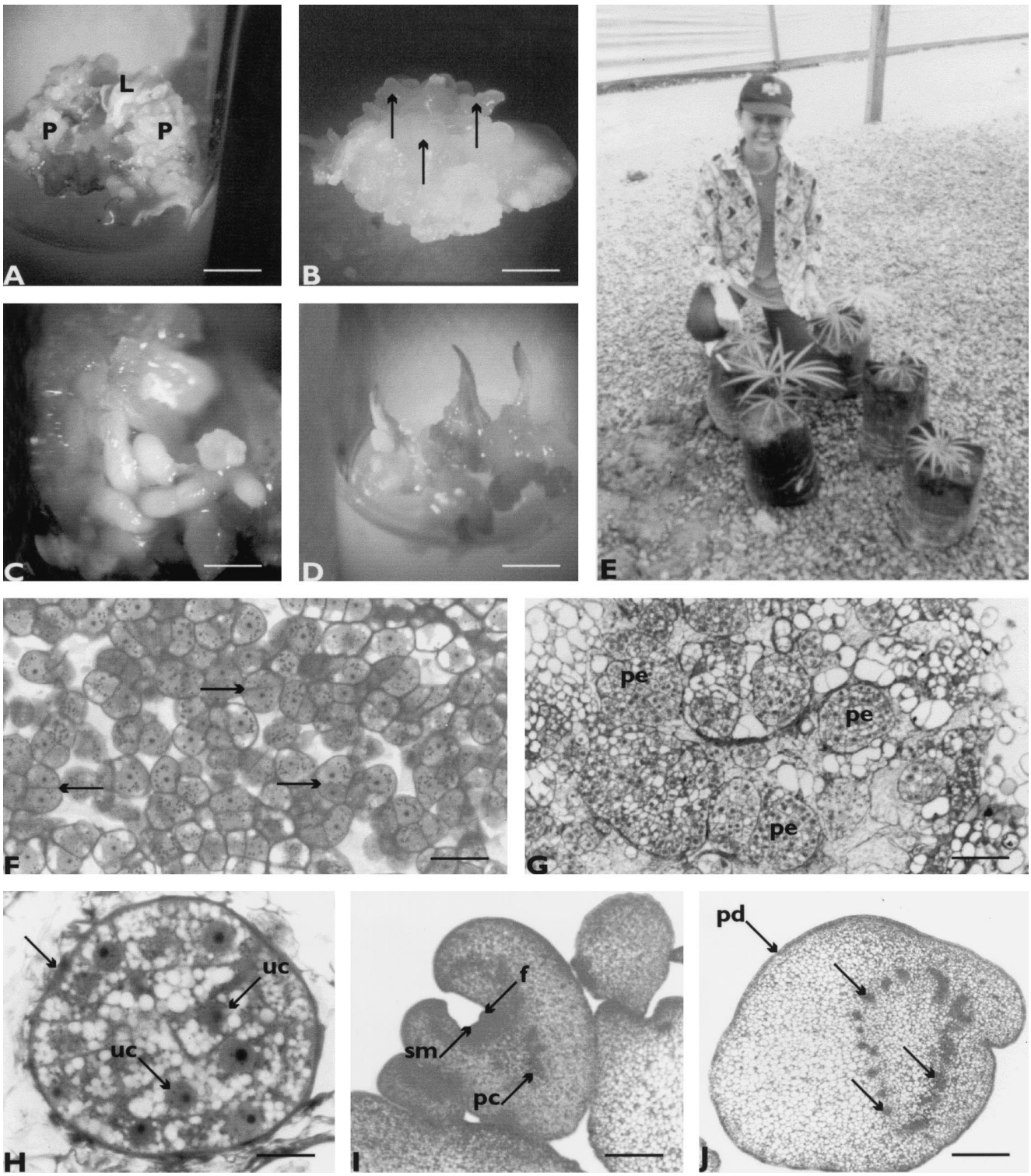

FIG. 2. *C. merrillii*. A, Proembryos (P) arising from an embryogenic callus produced by a leaf explant (L) (*bar* = 4.4 mm). B, Different types of glossy and translucent embryo-like structures (arrows) emerging from the same callogenic clump (*bar* = 4.7 mm). C, Germinating somatic embryos (*bar* = 4.4 mm). D, Somatic embryos developing a greenish shoot (*bar* = 4.4 mm). E, Somatic embryo-issued plants in the nursery ready to be field planted. F, Embryogenic cells dividing actively to form clusters (arrows) (*bar* = 31 μm). G, Proembryos (pe) at different stages of development (*bar* = 95 μm). H, Globular proembryo consisting of undifferentiated cells (uc) largely vacuolated and with thin walls, except at the periphery of the proembryo (arrow) (*bar* = 13.2 μm). I, Longitudinal section of a somatic embryo showing the shoot apical meristem (sm), a foliar initium (f) and the procambium (pc) (*bar* = 286 μm). J, Cross-section of a somatic embryo cotyledon showing the vascular bundles (arrows) and the protoderm (pd) (*bar* = 298 μm).

necrotic cells and cells with a high level of polyphenolic compounds increased, and the calluses began to degenerate. In contrast, friable and more yellowish calluses appeared to arise from a mixture of undifferentiated cells (small nucleolus and highly vacuolated) and embryogenic cells characterized by enlarged nucleoli, high nucleoplasmic ratio, dense little vacuolated cytoplasm and starch reserves in the form of granules (Fig. 1G). These embryogenic cells were gradually isolated from each other and often appeared to be surrounded by a polysaccharidic mucilage, stained pink by PAS, and which originated from the modification of the median lamella and the primary cell walls. This is generally an indication of healthy cells with prospects for further development.

These isolated embryogenic cells actively divided to form clusters. When transferred onto media with decreasing concentration of picloram, these became progressively 'embryonic' and could be easily distinguished by the presence of enlarged nucleoli and intense staining of the cytoplasm containing abundant soluble proteins. Concomitantly, starch granules disappeared. These clusters of embryonic cells delimited into zones that later evolved into proembryos (Fig. 1H). No protein reserves could be seen using NBB. Proembryos at the globular stage consisted of undifferentiated cells with uniformly thin walls except in the peripheral region where cell walls appeared thicker, isolating progressively these structures from surrounding degenerating cells. At this juncture, the development of the proembryos appeared to be quite heterogeneous, ranging from a degenerating process to different stages of maturation (Fig. 1I). Maturation corresponded to the formation of a protoderm through the establishment of a peripheral zone consisting of one and then several layers of cells. Vascular tissues then developed between shoot and root apices which were formed at each extremity, giving rise to a bipolar embryo (Fig. 1J).

Calamus merrillii. In contrast with *C. subinermis*, the three types of explants started to swell before they underwent callus formation within 6 wk after inoculation (Table 1). However, although 74% of the root explants produced calluses, no embryogenic structures were obtained. The root-derived calluses eventually became translucent or brownish due to phenolic oxidation after subsequent transfers and then decayed. Six weeks after having been inoculated under the same culture conditions, 90% and 84% of the zygotic embryos and young leaf portions used as explants, respectively, gave rise to friable calluses, without undergoing a soft watery phase as observed in *C. subinermis*. The friable calluses later became yellowish. Subculturing these calluses several times at 4–8-wk intervals onto the maturation medium with a lower concentration of picloram ranging from 4.1–20.7 μM promoted embryo formation, without noticeable difference between cultures derived from zygotic embryos or leaf portions. About 60% and 40% of the total zygotic embryo and leaf-derived calluses, respectively, became embryogenic.

As in *C. subinermis*, several types of proembryogenic structures could be distinguished within the same callogenic clump (Fig. 2A). Some were irregularly shaped, elongated or globular, but in general these structures were succulent in texture and translucent in appearance (Fig. 2B). Again, the most promising embryos were dome-shaped with a pointed apex that eventually developed into a greenish shoot (Fig. 2C, D). In some cases, callogenic clumps consisted of embryos tightly attached together. Separation of these structures resulted in their death as indicated by browning and decay of the tissues. Similarly, attempts to elongate the shoot-like apices using gibberellic acid (2.9 μM) gave results as observed for *C. subinermis*, that is, a deep greenish coloration without elongation and profuse rooting from the clusters. Overall, conversion was easier than for *C. subinermis* and successfully acclimatized somatic embryo-derived plantlets (Fig. 2E) were eventually established in field trials.

Histologically, somatic embryogenesis in *C. merrillii* presents a lot of similarities with what has been described for *C. subinermis.* Calluses produced by zygotic embryos and leaf portion explants were also derived from perivascular tissues. Embryogenic cells of friable and yellowish calluses, surrounded by a polysaccharidic mucilage and actively dividing (Fig. 2F), successively gave rise to proembryogenic clumps when transferred onto media with lower picloram concentrations. These evolved soon into clusters of embryonic cells corresponding to the globular proembryos observed in culture (Fig. 2G), and consisted of undifferentiated cells that did not exhibit any starch nor protein storage reserves when stained by PAS or NBB. These cells possessed thin walls except at the periphery of the proembryos (Fig. 2H). The next stage consisted in the differentiation of tissues such as the protoderm, the shoot apex, procambium, vascular tissues which were also visible in the cotyledons, and at the distal end, the root apex (Fig. 2I, J). Some somatic embryos displayed structural abnormalities such as multiple shoot apices. Cases of secondary somatic embryogenesis could be observed at the periphery of somatic embryos obtained from zygotic embryo primary explants, also amenable to adventitious organogenesis although no vascular connection could be clearly seen on the samples observed (data not shown).

Discussion

In vitro somatic embryogenesis studies in palms have so far been mostly devoted to oil palm (Jones and Hugues, 1989; Rival, 2000), date palm (Tisserat, 1987; Bhaskaran and Smith, 1995), and more recently to coconut (Verdeil et al., 1994; Blake and Hornung, 1995). As far as rattan species are concerned, this is the first histological study demonstrating the possibility of regenerating under *in vitro* conditions *C. merrillii* and *C. subinermis* through somatic embryogenesis, as recently established for *C. manan* (Goh et al., 1999).

The protocols applied for inducing somatic embryogenesis from organized tissues via callus formation are very similar to the procedures used for various species (Krikorian, 1989), especially with regard to exogenous auxin exposure (Brackpool et al., 1986; Thorpe, 1995). However, on *C. subinermis* and *C. merrillii*, just as for *C. manan* (Goh et al., 1999) and similarly to Benbadis' observation on date palm (personal communication), picloram was more efficient than 2,4-dichlorophenoxyacetic acid (2,4-D), the most commonly used auxin for inducing somatic embryogenesis in palms although at much higher concentrations (Reynolds and Murashige, 1979; Tisserat, 1987; Krikorian, 1989). The range of picloram concentrations tested did not have any significant effect on callus formation among explants of the same origin, consistent with other reports (Verdeil et al., 1994; Goh et al., 1999). The fact that when exposed to the same *in vitro* conditions, primary explant capacity to induce embryogenic calluses could vary according to the species has already been observed (Brackpool et al., 1986; Pannetier and Buffard-Morel, 1986), even though here it concerned species within the same genus. The higher morphogenetic potential

of *C. merrillii* explants to produce embryogenic calluses, and then somatic embryos including possible secondary embryogenesis, multiple apical meristems and also adventitious organogenesis at higher frequencies and faster than *C. subinermis* when exposed to the same *in vitro* protocols might be associated with *C. merrillii* organogenic features. Naturally, *C. merrillii* produces profusely multiple stems and lateral shoots, whereas *C. subinermis* is more prone to produce a single stem, similarly to the solitary *C. manan* in which root tips were also observed to be the most responsive explants for producing somatic embryos (Goh et al., 1999).

Classically, callus formation originated from perivascular tissues. Histological observations confirmed that soon embryogenic calluses or proembryogenic masses can be distinguished from non-embryogenic ones based on their external aspects (Krikorian, 1989). The wide diversity of cell types within proembryogenic calluses, from degenerating ones to proembryonic clusters, may be partly induced by unadapted culture medium composition. This aspect, as well as the influence of the genotype on proembryo formation, undoubtedly deserves to be further investigated considering the efforts needed for improving the process in other species (Tisserat, 1987; Krikorian, 1989; Rival, 2000). In the given experimental conditions, our histological observations support the unicellular origin of somatic embryos obtained for the two species. It appeared unambiguously that the proembryos were derived from segmenting single cells, similarly to what could be observed in *C. manan* (Goh et al., 1999), and also by Verdeil et al. (1994) in coconut. This unicellular-origin constitutes a striking illustration of the cell totipotency concept (Thorpe, 1995), in addition to the prospect for genetic engineering.

According to Merkle et al. (1995), disruption of tissue integrity resulting in the isolation of different types of cell which became less influenced by surrounding tissues may stimulate the induction of the 'competent state' (Krikorian, 1989). This differed radically from many palm species, including oil palm, where somatic embryogenesis was reported to be of multicellular origin (Schwendiman et al., 1988), notwithstanding that tissue culture conditions may have a determining influence on the uni- or multicellular origin of *in vitro* somatic embryos (Haccius, 1978; Michaux-Ferrière et al., 1992). According to Haccius (1978), certain unicellular-derived proembryos could give rise to secondary embryos via proembryonal cell complex. The secondary somatic embryos observed in *C. merrillii* may thus be of multicellular origin and embryogenic cultures may increase this phenomenon. The subsequent developmental stages of the proembryos are consistent with what are described for *C. manan* (Goh et al., 1999) and for other palms (Tisserat, 1987; Verdeil et al., 1994), but differ from coniferous and dicotyledonous somatic embryos (Merkle et al., 1995). Subsequently, lower levels of auxin are required for bipolar growth establishment (Merkle et al., 1995). The occurrence of aberrant growth patterns such as disoriented development, abnormal leaf curling and swelling more frequently seen in *C. subinermis*, has also been reported in many palms, especially in oil palm where somaclonal variations remain a serious concern (Krikorian, 1989; Rival, 2000).

Although this work resulted ultimately in the production of somatic embryo-derived plantlets for *C. merrillii* and to a lesser extent for *C. subinermis*, more advanced studies are needed for improving the process on a more reliable and efficient basis, especially when starting from mature selected plants that are growing outdoors. This approach obviously deserves overriding consideration for further genetic improvement programs (Goh et al., 1997).

References

Aziah, M. Y.; Manokaran, N. Seed and vegetative propagation of rattans. In: Proceedings of the Rattan Seminar, Kuala Lumpur, October 2–4 1984; 1985:13–21.

Bhaskaran, S.; Smith, R. H. Somatic embryogenesis in date palm (*Phoenix dactylifera* L.). In: Jain, S. M.; Gupta, P. K.; Newton, R. J., eds. Somatic embryogenesis in woody plants. Dordrecht, Boston, London: Kluwer Academic Publishers; 1995:461–470.

Blake, J.; Hornung, R. Somatic embryogenesis in coconut (*Cocos nucifera* L.). In: Jain, S. M.; Gupta, P. K.; Newton, R. J., eds. Somatic embryogenesis in woody plants. Dordrecht, Boston, London: Kluwer Academic Publishers; 1995:327–340.

Brackpool, A. L.; Branton, R. L.; Blake, J. Regeneration in palms. In: Vasil, I. K., ed. Cell culture and somatic cell, genetics of plants, vol. 3. Plant regeneration and genetic variability. London: Academic Press; 1986:207–222.

Dransfield, J.; Manokaran, N. Rattans. Plant resources of South-East Asia. No. 6. Wageningen: Pudoc Scientific Publishers; 1993.

Fernando, E. S. Diversity and conservation status of the Philippine rattans. RIC Bull. (2):9:7–21; 1990.

Fisher, D. B. Protein staining of ribboned epon sections for light microscopy. Histochemie 16:92–96; 1968.

Goh, D. K. S.; Bon, M. C.; Monteuuis, O. Prospects of biotechnology for a rattan improvement programme. Bois et Forêts des Tropiques 254:51–67; 1997.

Goh, D. K. S.; Michaux-Ferrière, N.; Monteuuis, O.; Bon, M.-C. Evidence of somatic embryogenesis from root tip explants of the rattan *Calamus manan*. In Vitro Cell. Dev. Biol. Plant 35:424–427; 1999.

Haccius, B. Question of unicellular origin of non-zygotic embryos in callus cultures. Phytomorphology 28:74–87; 1978.

Jones, L. H.; Hugues, W. A. Oil palm (*Elaeis guineensis* Jacq.). In: Bajaj, Y. P. S., ed. Biotechnology in agriculture and forestry, vol. 5. Trees II. Berlin, Heidelberg, Germany: Springer-Verlag Publishers; 1989:176–202.

Krikorian, A. D. The context and strategies for tissue culture of date, African oil and coconut palms. In: Dhawan, V., ed. Applications of biotechnology in forestry and horticulture. New York: Plenum Press; 1989:119–144.

Martoja, R.; Martoja, M. Initiation aux techniques de l'histologie animale. Paris: Masson et Cie Eds; 1967.

Merkle, S. A.; Parrott, W. A.; Flinn, B. S. Morphogenic aspects of somatic embryogenesis. In: Thorpe, T. A., ed. In vitro embryogenesis in plants. Dordrecht: Kluwer Academic Publishers; 1995:155–203.

Michaux-Ferrière, N.; Grout, H.; Carron, M. P. Origin and ontogenesis of somatic embryos in *Hevea brasiliensis* (Euphorbiaceae). Am. J. Bot. 79(2):174–180; 1992.

Murashige, T.; Skoog, F. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. 15:473–497; 1962.

Nasi, R.; Monteuuis, O. Un nouveau programme de recherches au Sabah: le rotin. Bois et Forêts des Tropiques 232:15–25; 1992.

Pannetier, C.; Buffard-Morel, J. Coconut palm (*Cocos nucifera* L.). In: Bajaj, Y. P. S., ed. Biotechnology in agriculture and forestry, vol. 1. Trees I. Berlin, Heidelberg, Germany: Springer-Verlag Publishers; 1986:430–450.

PCARRD. The Philippines recommends for rattan production. Los Banos: Philippine Council for Agriculture, Forestry and Natural Resources Research and Development (PCARRD); 1991.

Reynolds, J. F.; Murashige, T. Asexual embryogenesis in callus cultures of palms. In Vitro 15(5):383–387; 1979.

Rival, A. Somatic embryogenesis in oil palm. In: Jain, S. M.; Gupta, P. K.; Newton, R. J., eds. Somatic embryogenesis in woody plants, vol 6. Dordrecht, Boston, London: Kluwer Academic Publishers; 2000:249–290.

Schwendiman, J.; Pannetier, C.; Michaux-Ferrière, N. Histology of somatic embryogenesis from leaf explants of the oil palm *Elaeis guineensis*. Ann. Bot. 62:43–52; 1988.

Thorpe, T. A. In vitro embryogenesis in plants. Dordrecht: Kluwer Academic Publishers; 1995.

Tisserat, B. Palms. In: Bonga, J. M.; Durzan, D. J., eds. Cell and tissue culture in forestry, vol. 3. Dordrecht: Martinus Nijhoff Publishers; 1987:338–356.

Umali-Garcia, M. Tissue culture of some rattan species. In: Proceedings of the Rattan Seminar, Kuala Lumpur, October 2–4 1984; 1985:23–31.

Umali-Garcia, M.; Canlas-Mendoza, A. *In vitro* culture of rattan in the Philippines: progress, problems and prospects. In: Rao, A. N., Rao, R. eds. Proceedings of Training Courses cum Workshops: Rattan - taxonomy, ecology, silviculture, conservation, genetic improvement and biotechnology. Sarawak and Sabah, April 14–26, 1996:179–184.

Umali-Garcia, M.; Villena-Sanches, E. Tissue culture of rattan: progress and prognosis. In: Proceedings of the National Symposium/Workshop on Rattan, Cebu City, June 1–3 1988, PCARRD Book series no. 99; 1990:98–102.

Verdeil, J.-L.; Huet, C.; Grosdemange, F.; Buffard-Morel, J. Plant regeneration from cultured immature inflorescences of coconut (*Cocos nucifera* L.): evidence for somatic embryogenesis. Plant Cell Rep. 13:218–221; 1994.